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http://dx.doi.org/10.1289/ehp.1206333

Received: 30 November 2012
Accepted: 19 July 2013
Advance Publication: 23 July 2013

NIH National Institute of Environmental Health Sciences

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Running title: Bulky DNA adducts and birth weight

Acknowledgments

The NewGeneris (Newborns and Genotoxic exposure risks) study was funded by the European Union (EU Contract FOOD-CT-2005-016320). The study was also supported by the National Institute for Health Research, United Kingdom (Programme grant RP-PG-0407-10044), the Norwegian Ministry of Health, the Norwegian Ministry of Education and Research, the Norwegian Research Council/FUGE (Grant 151918/S10), the EU funded HiWATE (Contract Food-CT-2006-036224), the U.S. National Institutes of Health (NIH)/National Institute of Environmental Health Sciences (Contract NO-ES-75558) and the U.S. NIH/National Institute of Neurological Disorders and Stroke (Grant 1 UO1 NS 047537-01). M.P. holds a Juan de la Cierva post-doctoral fellowship awarded from the Spanish Ministry of Science and Innovation (JCI-2011-09479). We thank participants in the study and the collaborators from the hospitals.

Conflict of interest statement

The authors and collaborators declare that they have no actual or potential competing financial interests.

Abstract

Background: Tobacco-smoke, airborne, and dietary exposures to polycyclic aromatic hydrocarbons (PAHs) have been associated with reduced prenatal growth. Evidence from biomarker-based studies of low-exposed populations is limited. Bulky DNA adducts in cord blood reflect the prenatal effective dose to several genotoxic agents including PAHs.

Objectives: We estimated the association between bulky DNA adduct levels and birth weight in a multicenter study and examined modification of this association by maternal fruit and vegetable intake during pregnancy.

Methods: Pregnant women from Denmark, England, Greece, Norway, and Spain were recruited in 2006-2010. Adduct levels were measured by the ^{32}P-postlabelling technique in white blood cells from 229 mothers and 612 newborns. Maternal diet was examined through questionnaires.

Results: Adduct levels in maternal and cord blood samples were similar and positively correlated (median, 12.1 vs. 11.4 adducts in 10^8 nucleotides; Spearman rank correlation coefficient=0.66, $p<0.001$). Cord blood adduct levels were negatively associated with birth weight, with an estimated difference in mean birth weight of -129 g (95% CI: -233, -25) for infants in the highest versus lowest tertile of adducts. The negative association with birth weight was limited to births in Norway, Denmark, and England, the countries with the lowest adduct levels, and was more pronounced in births to mothers with low intake of fruit and vegetables (-248 g; 95% CI: -405, -92) compared to those with high intake (-58 g; 95% CI: -206, 90)

Conclusions: Maternal exposure to genotoxic agents that induce the formation of bulky DNA adducts may affect intrauterine growth. Maternal fruit and vegetable consumption may be protective.

Introduction

Environmental exposures *in utero* may have adverse effects on health both immediately and in later life. Measurement of biomarkers in cord blood improves exposure assessment and may improve our understanding of biological mechanisms during this critical window of exposure and vulnerability (Wild and Kleinjans 2003).

Bulky DNA adducts are a widely accepted and sensitive biomarker of the biologically effective dose of genotoxic agents in complex environmental exposures, including those in ambient air, tobacco smoke, and diet (Godschalk et al. 2005; Karttunen et al. 2010; Kovács et al. 2011). They reflect individual exposure, absorption, and metabolic activation of heterogeneous adduct-forming compounds, in combination with the ability to repair induced DNA damage (Farmer 1994), and may be predictive of cancer risk (Veglia et al. 2008).

Bulky DNA adducts are commonly detected in human DNA by ^{32}P-postlabelling combined with multi-dimensional thin-layer chromatography. Among common environmental genotoxic agents, polycyclic aromatic hydrocarbons (PAHs) (IARC 2010) cause DNA damage that is readily detectable as bulky DNA adducts, though the chemical nature of the DNA damage that leads to adduct formation is not known with certainty. A positive correlation between DNA adducts in blood and PAH exposure has been reported in adult populations exposed to high levels of PAHs in ambient air or food (Nielsen et al. 1996; van Maanen et al. 1994), which suggests that bulky DNA adducts reflect DNA damage caused by genotoxic PAHs. Bulky DNA adducts and more specific PAH-related DNA adducts have been detected in human umbilical cord white blood cells (Hansen et al. 1993; Godschalk et al. 2005; Pedersen et al. 2009; Perera et al. 1998, 2003,

2005; Topinka et al. 2009), in human placenta (Everson et al. 1988; Hansen et al. 1993; Sram et al. 2006) and in *ex vivo* human placental perfusions (Karttunen et al. 2010), which suggests that PAHs and other environmental genotoxic agents are capable of forming DNA adducts *in utero*.

Intake of meat with a blackened surface (Pedersen et al. 2012a), exposure to traffic-related air pollution during pregnancy (Pedersen et al. 2009) and smoking during pregnancy (Hansen et al. 1993, Godschalk et al. 2005; Pedersen et al. 2009) have been associated with higher levels of bulky DNA adducts in human cord blood. However, evidence regarding associations between bulky DNA adducts and birth outcomes is conflicting. Smoking-related DNA adducts measured by the ^{32}P-postlabelling method in placental tissue from 30 women in the US were associated with reduced birth weight (Everson et al. 1988), but there were no associations between bulky DNA adducts in placenta tissue from 199 women in the Czech Republic and birth weight, the risk of low birth weight (<2500 g), gestational duration, or preterm delivery (Sram et al. 2006).

Fruit and vegetable consumption is considered beneficial for health (Slavin and Lloyd 2012) and may protect against cancer [Word Cancer Research Fund (WCRF) 2007] through antioxidative effects and other properties related to dietary intake of fiber, folate, and other beneficial nutrients. In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study higher intake of fibers was negatively associated with bulky DNA adduct levels in white blood cells from 1,085 adults (Peluso et al. 2008). In addition, a diet rich in vitamin C has been associated with lower levels of DNA damage (as reviewed by Sram et al. 2012) and high maternal vitamin C intake during pregnancy appeared to reduce the association between estimated dietary benzo[a]pyrene (B(a)P) intake and size at birth of 586 newborns from Spain (Duarte-Salles et al. 2012).

In the present study we investigated the association between bulky DNA adduct levels and birth weight in 612 newborns and further assessed whether maternal consumption of fruit and vegetables during pregnancy modified this association.

Methods

Study population. The study was conducted as a part of the NewGeneris study of the impact of diet during pregnancy on child health (Merlo et al. 2009). Pregnant women were enrolled during 2006 to 2010 from eleven maternity units located in Copenhagen, Denmark; Bradford, England; Heraklion, Greece; Oslo, Norway; and Barcelona and Sabadell, Spain (Pedersen et al. 2012b). Births were included in the present analysis if they occurred during the periods of cord blood collection and processing, there was a sufficient volume of cord blood, and blood processing and biomarker analysis was successful. Precise participation rates for the present analysis cannot be estimated because the number of births that might have been eligible cannot be determined.

Detailed information on personal characteristics was obtained from the mothers by using extensive questionnaires collected before or around the time of delivery (Table 1). The questionnaires were self-administered (Denmark-2009, Norway), partly supported (Denmark-2007, Spain and England) and administered by an interviewer (Greece) (Pedersen et al. 2012b). Dietary information concerning diet during pregnancy was obtained from country-specific food-frequency questionnaires. Information on birth weight, birth head circumference, gestational age, sex, and mode of delivery was obtained from maternity records. Gestational age at birth was based on last menstrual period and/or ultrasound-based estimated date of conception and corrected by ultrasound measurements if there was a discordance of seven days or more between both estimates

Cord blood DNA adduct measurements were available from 630 newborns born to women with singleton deliveries. We excluded 18 newborns with missing information on maternal smoking, gestational age, birth weight, and/or sex, and included 612 newborns.

Ethical approval was obtained from the ethics committee in each country. Written informed consent was obtained from all participating women.

Blood collection and bulky DNA adduct analysis (^{32}P-postlabelling). Umbilical cord blood (~ 50 mL) was collected immediately after delivery. Peripheral blood (~ 45 mL) was also drawn from 229 mothers.

DNA was isolated centrally from ~ 0.5 mL aliquots of buffy-coat samples using Qiagen Midi Kit Cat. No.13343 (Qiagen, Hilden, Germany) with some modifications (Arab et al. 2009). Levels of bulky DNA adducts were determined by using the ^{32}P-postlabelling method with the nuclease P1 adduct enrichment version according to standardized protocols (Godschalk et al. 2005; Karttunen et al. 2010; Kovács et al. 2011), that were harmonised and adjusted in an inter-laboratory comparison study among the three ^{32}P-postlabelling investigator laboratories, including the use of the same external benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE)-DNA standard (111 adducts in 10^8 normal nucleotides (nt), which was a kind gift from Dr. Frederick F. Beland (NCTR, Arkansas, USA)). All samples from Greece, Spain, Norway and the Danish samples collected in 2006-2007 were analysed in Budapest, Hungary (61% of the samples), the Danish samples from 2009 were analysed in Stockholm, Sweden (21%) and the samples from England in Maastricht, the Netherlands (18%). The inter-laboratory comparison study showed a very high repeatability between two of the three laboratories while the adduct levels measured in the third laboratory were consistently 3.7 times lower than the mean levels determined by the two other laboratories.

Differences in DNA adduct determinations between laboratories are normally observed due to the complicated multi-step and sensitive procedures used for the detection of the adducts, and interlaboratory studies are, therefore, necessary. A correction for the laboratory factor of 3.7 was thus applied to the samples analysed in Stockholm (Denmark 2009, 21% of the total). A sensitivity analysis that included or subsequently excluded these samples gave similar results and, therefore, all analyses were based on the total study population including the corrected data.

The individual level of DNA adducts was obtained as the average of at least two independent measurements. The detection limit of the assay was approximately 0.1-0.3 adducts per 10^8 unmodified nucleotides (n/10^8 nt).

Statistical analysis. Linear regression models with a random effect for country were performed to estimate the difference in mean birth weight (g) associated with bulky DNA adduct levels in cord blood. Furthermore, we estimated associations with head circumference (cm) and gestational age (completed weeks) at birth. Low birth weight (<2,500 g, n=7) and preterm (<37 completed weeks of gestation, n=26) were too uncommon to estimate associations with bulky DNA adduct levels.

DNA adduct levels measured in cord blood were modelled as categorized according to tertiles as low (n=205), middle (n=203), or high (n=204) (<5.9, ≥5.9 – 12.4, and ≥12.5 adducts/10^8 nt, respectively).

We examined the effect of different degrees of adjustment for potential confounders on the association of bulky DNA adduct levels on birth weight. Potential confounders selected *a priori* for the adjusted model were gestational age (completed weeks, continuous), sex, maternal pre-pregnancy body mass index (BMI, kilograms per meter squared), parity (0, 1+), maternal age

(years), maternal ethnicity (white, non-white), self-reported maternal active smoking at the end of pregnancy (no, yes), self-reported maternal exposure to second hand smoke (SHS) during pregnancy (no, yes), ethylene oxide-hemoglobin (Hb) adduct levels in cord blood (pmol/g Hb; to assess exposure to tobacco smoke during pregnancy), mode of delivery (vaginal, Caesarean section), dietary supplements (none, any), maternal consumption of fruit and vegetables during pregnancy (low, high), season of delivery (March-May, June-August, September-November, December-February), country of delivery, and maternal education (low, middle, high) as a marker of socio-economic position. In addition, we estimated basic adjusted associations using models that included country, maternal smoking at the end of pregnancy, sex, and gestational age only.

Given that bulky DNA adduct levels in cord blood reflect a steady state between DNA damage and repair during the previous few months (Godschalk et al. 2005), we classified women who smoked during the last four months of pregnancy as “smokers”, while those who never smoked or who quit before the last four months of pregnancy were classified as “non-smokers”. Women exposed to SHS in the home and/or elsewhere during pregnancy were categorized as “exposed”. In addition, to further assess exposure to dietary acrylamide and tobacco smoke during pregnancy, we adjusted for acrylamide- and ethylene oxide hemoglobin (Hb) adduct levels (pmol/g Hb), respectively, in cord blood samples (von Stedingk et al. 2011).

Maternal intakes of fruit and vegetables during pregnancy (g/day, based on 20 to 61 questionnaire items depending on country) were categorized as high or low according to overall and country-specific median levels as a proxy measure of the consumption of nutrients that might be protective against genotoxic activation processes. We also classified mothers according to their intake of fruit high in vitamin C and other antioxidants (i.e. all types of citrus fruits, both

in terms of whole fruits and juice, kiwi fruit and berries, ranging from three to seven questionnaire items). Women (n=54) with a total energy estimate of <500 or >6,000 kcal/day were excluded from adjusted analyses and analyses of effect modification by diet (Butte and King 2005; Willett 1998).

In addition, we performed a meta-analysis by country to derive country-specific effect estimates of associations between cord blood DNA adduct levels and birth weight. Pregnancy outcomes in Northern European countries (England, Denmark, and Norway, n=367), which had low average levels of adducts, were compared with outcomes in Southern European countries (Greece and Spain, n=245), which had higher average adduct levels.

To estimate the association between bulky DNA adduct levels and term birth weight, we repeated the main analysis after excluding preterm deliveries (n=26). We used an alpha level of 5% for statistical significance. All statistical analyses were performed using Stata S.E. version 12.1 (StataCorp, Texas, USA).

Results

Study population. The study population was composed of neonates from Denmark (33%), Spain (29%), England (18%), Greece (11%) and Norway (10%). Mothers were predominately white Europeans, multiparous, and non-smoking (Table 1). The children were mainly born at term (96%) and weighed more than 2,500 g at birth (97%). Some study population characteristics differed significantly between the Northern and Southern European populations, e.g. maternal smoking (8% versus 18%, respectively) while characteristics such as maternal pre-pregnancy BMI and dietary supplement use were similar. The daily median fruit and vegetable intake of the

Southern European mothers was 58 g higher than that of Northern European mothers, but the difference was not statically significant (p=0.13). The difference in fruit and vegetable intake between Southern and Northern European mothers was also not statically significant after adjustment for individual total energy intake. Differences were smaller for fruits high in vitamin C (Table 1).

Adduct levels in maternal and cord blood samples. All maternal (n=229) and cord blood (n=612) samples had detectable levels of adducts. Median levels of adducts in paired maternal and cord blood samples were similar (12.1 vs. 11.4 adducts/10^8 nt, p=0.23). Cord blood adduct levels ranged from 0.6 to 87.5 (adducts/10^8 nt) and were significantly positively correlated with maternal levels (Spearman's rank correlation coefficient=0.66, p=0.001, n=229).

Bulky DNA adduct levels were higher in children from Southern Europe (median 12.8/10^8 nt, range 0.8–87.5), than from Northern Europe (median 7.0/10^8 nt, range 0.6–52.7; p<0.001) while an opposite pattern was observed for birth weight (medians of 3325 g and 3544 g, respectively, p<0.001) (Table 1, Figure 1). The difference in DNA adduct levels was also observed when the analysis was restricted to children born to mothers who did not smoke during the last 4 months of pregnancy (median 13.0/10^8 nt, range 0.8-87.5 for Southern Europe vs. 6.8/10^8 nt, range 0.6-52.7 for Northern Europe; p<0.001). Median adduct levels in the children of non-smokers also differed significantly (p<0.001) among the individual countries (Greece 13.4/10^8 nt, range 0.8-43.9, n=54; Spain 12.9/10^8 nt, range 1.1-87.5, n=148; England 9.5/10^8 nt, range 0.6-52.7, n=57; Denmark 6.4/10^8 nt, range 1.3-42.7, n=192; Norway 5.4/10^8 nt, range 1.2-22.3, n=57). The same pattern of higher adduct levels in children from Southern Europe than those from Northern Europe was found for children born to non-smokers without exposure to second hand smoke.

The median bulky DNA adduct level in the 71 children born to mothers who actively smoked at the end of their pregnancy (10.9 adducts/10^8 nt, range 0.6–73.9) was higher than the median level in the 541 children of mothers who never smoked or quit before the last four months of pregnancy (8.2 adducts/ 10^8 nt, , range 0.6–87.5, p=0.07). Adduct levels were lowest in the 505 children born to mothers who never smoked during their pregnancy (8.0 adducts/10^8 nt, range 0.6–87.5, p=0.10).

Adduct levels in cord blood and birth outcomes. Higher levels of bulky DNA adducts in cord blood were associated with lower birth weight (Table 2). For the full study population (n=612), the estimated difference in mean birth weight for infants in the highest tertile versus the lowest tertile of adduct levels was -110 g (95% CI: -192, -28) based on basic adjusted models that included country, maternal smoking at the end of pregnancy, sex, and gestational age only. The corresponding association was similar when restricted to the 541 mothers who did not smoke during the last four months of pregnancy (-108 g; 95% CI: -202, -14), but was slightly stronger when restricted to the 505 mothers who did not smoke at any time during the pregnancy (-124 g; 95% CI: -216, -32).

After further adjusting for maternal age, pre-pregnancy BMI, exposure to SHS, maternal education, ethnicity, intake of fruit and vegetables, delivery type and season of delivery (n=409), the estimated difference in mean birth weight associated with the highest versus lowest tertile of DNA adduct levels was -129 g (95% CI: -233, -25) (Table 2). After additional adjustment for ethylene oxide-cord blood Hb adduct levels (pmol/g Hb, n=390), the estimated mean difference was -139 g (95% CI: -245, -32). When we further adjusted for acrylamide Hb adduct levels in cord blood (pmol/g Hb, n=390) the associations remained practically unchanged (-140 g (95% CI: -247, -34).

When 26 preterm births (<37 weeks) were excluded, the estimated difference in birth weight for infants in the highest versus lowest tertile of adduct levels was -139 g (95% CI: -245, -33).

Bulky DNA adduct levels were negatively associated with head circumference based on the basic adjusted model (-0.28 cm for the highest versus lowest tertile; 95% CI: -0.59, 0.03, p=0.08, n=530) and after further adjustment for maternal age, pre-pregnancy BMI, exposure to SHS, maternal education, ethnicity, intake of fruit and vegetables, delivery type and season of delivery (-0.33 cm; 95% CI: -0.72, 0.06, p=0.10, n=388).

The estimated difference in mean gestational age at birth for infants in the highest versus lowest tertile of adduct levels was -0.29 weeks (95% CI, -0.63, 0.04; p=0.08) based on the further adjusted model. When restricted to vaginal deliveries (n=243, further adjusted model) the estimated difference in gestational age was -0.54 weeks (95% CI: -1.06, -0.03), compared with a difference of 0.15 weeks (95% CI: -0.23, 0.53; p=0.43) for deliveries by Caesarean section (n=166).

Differences between countries. Consistent with associations estimated for the full study population, mean birth weight was significantly lower for Northern European infants in the highest versus lowest tertile of adduct levels (-119 g; 95% CI: -234, -4) based on the basic adjusted model. However, there was a non-significant positive association among the 245 Southern Europeans (71 g; 95% CI: -59, 202; p=0.28). Estimates based on adjusted models also indicated negative associations for the Northern European countries, but not for the Southern European countries (Figure 2, p-value for heterogeneity=0.03), and when imputed data were used to include individuals with missing information on covariates (results not shown). Therefore, although birth weight was lowest and adduct levels were highest in Greece and Spain

(Figure 1), the negative association between adduct levels and birth weight estimated for the full study population appears to be driven by the Northern countries.

Differences by intake of fruit and vegetables. The association of bulky DNA adduct levels with birth weight differed according to maternal intake of fruit and vegetables and intake of fruits high in vitamin C, although interactions were only marginally significant (Table 3). The estimated difference in birth weight between the highest and lowest tertiles of adduct levels was greater among births to mothers with low intakes of fruit and vegetables (-248 g; 95% CI: -405, -92) than among births to mothers with high intakes (-58 g; 95% CI: -206, 90; p=0.44). Consumption of dietary supplements during pregnancy was common in both Northern and Southern Europeans (85% and 90%, respectively) and did not appear to modify or confound associations between adduct levels and birth weight (data not shown).

Discussion

We measured levels of bulky DNA adducts in white blood cells from cord blood in a large multi-center European prospective general population study and found that higher adduct levels in cord blood were significantly negatively associated with birth weight. The negative association was observed among newborns from England, Denmark, and Norway who had the lowest average adduct levels, but was not evident among Southern Europeans, who had the highest mean adduct levels. The negative association with birth weight was stronger among the children of mothers with low versus high intakes of fruit and vegetables.

Tobacco smoke contains PAHs and other DNA adduct-forming compounds in addition to other potentially harmful compounds, and active maternal smoking is a recognized risk factor for

reduced fetal growth (Li et al. 1993). Furthermore, ambient airborne PAHs (Wilhelm et al. 2011) and intake of barbecued meat during pregnancy has also been associated with reduced birth weight (Jedrychowski et al. 2012).

The results of the previous similar biomarker-based studies on fetal growth are inconsistent; studies based on 1-hydroxypyrene in maternal urine (n=449, Poland) (Polanska et al. 2010), bulky DNA adducts (n=30, newborns of smokers, US) (Everson et al. 1988), and structurally related PAH-DNA adducts in cord blood (n=135, newborns of women living in coal-burning areas, Poland) (Perera et al. 1998) support an association between higher prenatal exposure to PAHs and reduced birth weight opposite to the findings from cord blood based studies on bulky DNA adducts (Sram et al. 2006), BPDE-DNA adducts (n=181, newborns of women who lived near the World Trade Center fires on 11 September 2001 while they were pregnant) (Perera et al. 2005) and on BPDE-DNA adducts (n=150, newborns of women living near a coal-fired power plant, China) (Tang et al. 2006). Results from these studies are, however, inconsistent and evidence from European populations exposed to contemporary lifestyle and environment is limited.

Mechanisms by which environmental genotoxicants that cause bulky DNA adduct formation might affect fetal growth are not known, but in along with direct modification of DNA (measurable as bulky DNA adducts), possible mechanisms may include binding to aryl hydrocarbon receptor (AhR) and/or other receptors causing endocrine disruption, altered placental growth, decreased placental exchange of nutrients and gases *in utero* possibly related to induction of P450 enzymes, global DNA methylation changes, induction of apoptosis, altered gene expression, cellular mutations, or oxidative stress (Baird et al. 2005; IARC 2010; Kannan et al. 2006; Sram et al. 2005; Tang et al. 2012). Developmental and reproductive toxicity due to

prenatal exposure to PAHs and similar AhR ligands has been observed in various animal species (Pocar et al. 2005; Sayal and Li 2007).

Our findings suggest that maternal intake of fruit and vegetables may modify the association between bulky DNA adduct levels and birth weight. This finding is consistent with the findings of a questionnaire-based study evaluating dietary benzo[a]pyrene (Duarte-Salles et al. 2012).

Children from Southern Europe had, on average, higher bulky DNA adduct levels and lower birth weight than Northern European children. However a negative association between adducts and birth weight was only found in the Northern Europeans. The same pattern of higher bulky DNA adduct levels in Southern Europe than in Northern Europe has been found in adults (Ricceri et al. 2010), and this may reflect differences in ambient air quality (Table 4), but also wider geographical differences in diet, food preparations and other factors, or perhaps, different susceptibility towards environmental genotoxic agents. It is possible that exposures that cause adducts in children from Northern Europe also cause reduced birth weight, while exposures responsible for adducts among Southern European children might differ and may not affect birth weight. A complementary explanation could involve a saturation effect of the toxicity of these adducts on birth weight, although other studies in populations exposed to very high air-pollution and corresponding high adduct levels have reported negative associations with birth weight (Perera et al. 1998, 2005).

A key strength of the present study is the measurement of bulky DNA adducts in cord blood, which enabled a more accurate evaluation of the biologically effective dose of genotoxic agents resulting from complex environmental exposures than estimates based solely on external exposure assessment. Large biomarker-based studies are rare due to their costs and complexity.

Standardized protocols were developed (Merlo et al. 2009) and applied to the collection of cord blood from multiple study centres. Detailed information on maternal characteristics and diet was collected in a manner that allowed pooling of data from five different countries (Pedersen et al. 2012b). In addition to increasing the sample size, enrolling participants from different countries allows us to test hypotheses in different settings.

^{32}P-postlabelling is considered to be most suitable and sensitive method for assessing total DNA damage resulting from exposures to unknown, complex mixtures of genotoxic compounds. We used the validated in vitro BPDE-DNA standard in each ^{32}P-postlabelling session in duplicate for normalization of the DNA adduct levels. Each DNA sample was analyzed at least twice. The inter-laboratory comparison study, the use of common protocols, and use of a validated BPDE-DNA standard minimized the potential for measurement error. Thus we believe that measurement error of the method was properly handled and is not a special concern in our study.

Low birth weight is an important outcome as it is associated with greater risk of neonatal mortality, hypertension and cardiovascular disease, diabetes, certain cancers, reduced and/or delayed postnatal growth, and cognitive development (Gluckman et al. 2008). Our findings suggest that environmental exposures that result in the *in utero* formation of bulky DNA adducts also may affect prenatal growth, and that this potential effect may be reduced by high maternal fruit and vegetable consumption.

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Table 1. Study population characteristics. -

Characteristics	All[a] N=612	North[b] N=367	South[b] N=245	p[c]
Country				<0.001
Greece	68 (11.1)	0 (0.0)	68 (27.8)	
Spain	177 (28.9)	0 (0.0)	177 (72.2)	
Norway	58 (9.5)	58 (15.8)	0 (0.0)	
England	109 (17.8)	109 (29.7)	0 (0.0)	
Denmark	200 (32.7)	200 (54.5)	0 (0.0)	
Maternal age (years)	32 (15-46)	32 (17-46)	31 (15-46)	<0.001
White European mother	473 (77.5)	319 (86.9)	154 (63.4)	<0.001
Maternal education[d]				<0.001
Low	113 (21.9)	59 (18.4)	54 (27.8)	
Middle	189 (36.7)	99 (30.8)	90 (46.4)	
High	213 (41.4)	163 (50.8)	50 (25.8)	
Multipara mother	390 (65.2)	240 (67.2)	150 (62.2)	0.21
Maternal BMI (kg/m^2)	22.8 (15.8-56.0)	22.7 (15.8-54.6)	23.1 (16.8-56.0)	0.24
Energy intake (kcal/d)	2457 (622-5918)	2480 (622-5918)	2402 (874-5844)	0.79
Fruit and veg. (g/d)	579 (0-5023)	557 (0-5023)	615 (0-3387)	0.13
Fruit and veg. (g/1000 kcal/d)	235 (0-1099)	221 (0-1065)	260 (0-1099)	0.02
Vitamin C fruit (g/day)	121 (0-1810)	120 (0-1122)	122 (0-1810)	0.26
Vitamin C fruit (g/1000 kcal/d)	50 (0-490)	48 (0-448)	52 (0-490)	0.35
Dietary supplement intake	436 (87)	271 (85)	165 (90)	0.18
Maternal active smoking[e]	71 (11.6)	28 (7.6)	43 (17.6)	<0.001
Second hand smoke[f]	213 (37.0)	97 (27.5)	116 (52.0)	<0.001
Ethylene oxide (pmol/g Hb)[g]	9.7 (0.5-120.7)	9.9 (0.5-120.7)	9.6 (2.6-88.1)	0.999
Season of delivery				<0.001
March-May	162 (26.5)	116 (31.6)	46 (18.8)	
June-August	90 (14.7)	50 (13.6)	40 (16.3)	
September-November	227 (37.1)	151 (41.1)	76 (31.0)	
December-February	133 (21.7)	50 (13.6)	83 (33.9)	
Vaginal mode of delivery	342 (56.0)	161 (43.9)	181 (74.2)	<0.001
Male	322 (52.6)	187 (51.0)	135 (55.1)	0.31
Gestational age (weeks)	39 (33-43)	39 (35-42)	39 (33-43)	<0.001
<37 weeks	26 (4.3)	5 (1.4)	21 (8.6)	
Birth weight (g)	3440 (2060-4700)	3544 (2060-4700)	3325 (2190-4510)	<0.001
<2,500 g	7 (1.1)	3 (0.8)	4 (1.6)	<0.001
Birth head circumference (cm)	35 (30-39)	35 (31-39)	35(30-38)	<0.001
Adducts ($n/10^8$ nucleotides)[g]	8.4 (0.6-87.5)	7.0 (0.6-52.7)	12.8 (0.8-87.5)	<0.001

Number of subjects (%) or Median (Min-Max) -

[a]Total in specific variables may be less to 612 because of missing values. [b]North refers to Denmark, England and Norway; South refers to Greece and Spain. [c]P-value from Chi-square or Kruskall-Wallis test for North-South comparisons. [d]Country-specific definition. [e]Women who smoked at end of pregnancy. [f]At home and elsewhere. [g]Measured in cord blood.

Table 2. Change in birth weight (g) associated with cord blood bulky DNA adduct levels. -

Variable	N	ß (95% CI)	p -
Basic adjusted[a]			
Adducts (increase of 10 adducts / 10^8 nt)	612	-30 (-62, 2)	0.07
Low (<5.9 / 10^8 nt)[b]	205	Ref.	
Middle (5.9-12.4 / 10^8 nt)	203	-47 (-128, 35)	0.26
High (>12.4 / 10^8 nt)	204	-110 (-192, -28)	0.009
Further adjusted[c]			
Adducts (increase of 10 adducts / 10^8 nt)	409	-21 (-62, 21)	0.32
Low (<5.9 / 10^8 nt)[d]	153	Ref.	
Middle (5.9-12.4 / 10^8 nt)	140	-51 (-146, 43)	0.29
High (>12.4 / 10^8 nt)	116	-129 (-233, -25)	0.015

Nt; nucleotides. Ref; reference.

[a]Effect estimates on birth weight (g) in linear regression models adjusted for gestational age, sex, maternal active smoking at the end of pregnancy and country (random effect). [b]The mean birth weight of the reference group was 3,510 g. [c]Further adjusted for maternal ethnicity, maternal pre-pregnancy BMI, parity, maternal age, maternal exposure to SHS, mode of delivery, maternal education, maternal consumption of fruit and vegetables and season of delivery. [d]The mean birth weight of the reference group was 3,559 g.

Table 3. Modification of the change in birth weight (g) associated with bulky DNA adduct levels by maternal intake of fruit and vegetables during pregnancy.

		Low maternal intake[a]			High maternal intake		
Models	N	ß[b] (95% CI)	p	N	ß (95% CI)	p	p[c]
Fruit and vegetables							
Adducts (increase of 10 adducts / 10^8 nt)	197	-22 (-80, 36)	0.45	212	-22 (-86, 42)	0.51	0.77
Low (<5.9 / 10^8 nt)[d]	71	Ref.		82	Ref.		
Middle (5.9-12.4 / 10^8 nt)	71	-78 (-217, 61)	0.27	69	-37 (-173, 100)	0.60	0.75
High (>12.4 / 10^8 nt)	55	-248 (-405, -92)	0.002	61	-58 (-206, 90)	0.44	0.077
Fruit high in vitamin C							
Adducts (increase of 10 adducts / 10^8 nt)	201	-39 (-94, 15)	0.15	208	3 (-64, 70)	0.93	0.63
Low (<5.9 / 10^8 nt)[e]	66	Ref.		87	Ref.		
Middle (5.9-12.4 / 10^8 nt)	73	-120 (-259, 19)	0.09	67	-0 (-129, 128)	1.00	0.54
High (>12.4 / 10^8 nt)	62	-266 (-421, -112)	0.001	54	-39 (-186, 107)	0.60	0.26

Nt; nucleotides. Ref; reference.

[a]Low corresponds to <579 g/d which is the overall median intake and high corresponds to ≥579 g/d in terms of fruit and vegetables. For fruit high in vitamin C low correspond to <121 g/d and high correspond to ≥121 g/d. [b]Effect estimates on birth weight (g) in linear regression models further adjusted, see Table 2. [c]P-value for the interaction term between maternal intake (low, high) and bulky DNA adduct level in cord blood. [d]The mean birth weight of the reference group was 3,613 g for low intake and 3,513 g for high intake. [e]The mean birth weight of the reference group was 3,593 g for low intake and 3,534 g for high intake.

Table 4. Annual mean of ambient air pollution ($\mu g/m^3$).

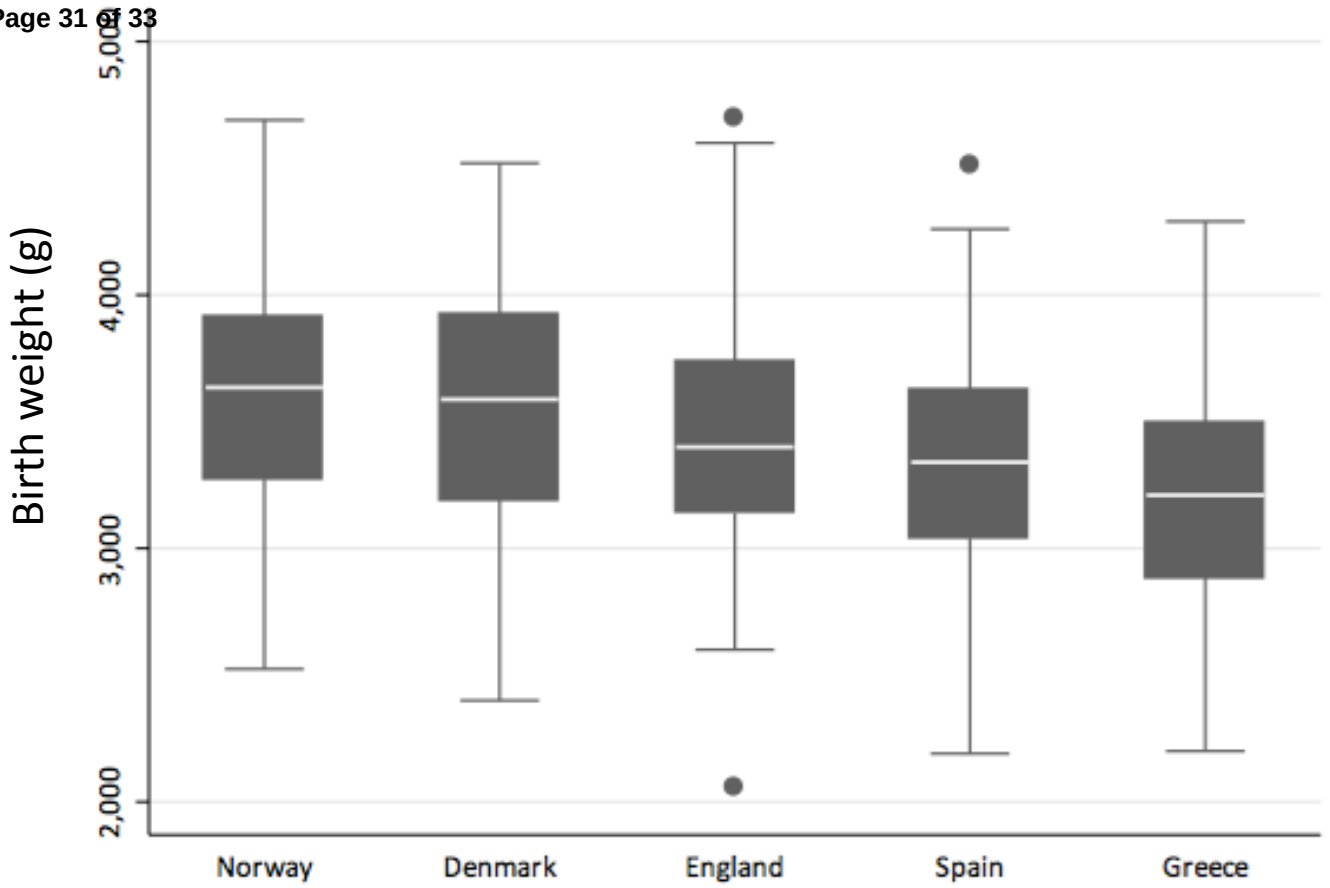
Location	Year	NO_2	PM_{10}	$PM_{2.5}$	Reference
Denmark, Copenhagen	2007	19	24	10	(NERI 2013)
Denmark, Copenhagen	2009	18	21	11	(NERI 2013)
Norway, Oslo and Akershus	2008	38	11	10	(NILU 2013)
England, Bradford	2008	25	n.a.	n.a.	(CBMDC 2009)
Spain, Sabadell	2007	29	40	18	(Gencat 2013)
Spain, Barcelona	2009	40	34	20	(Gencat 2013)
Greece, Heraklion	2007	41	20	n.a.	

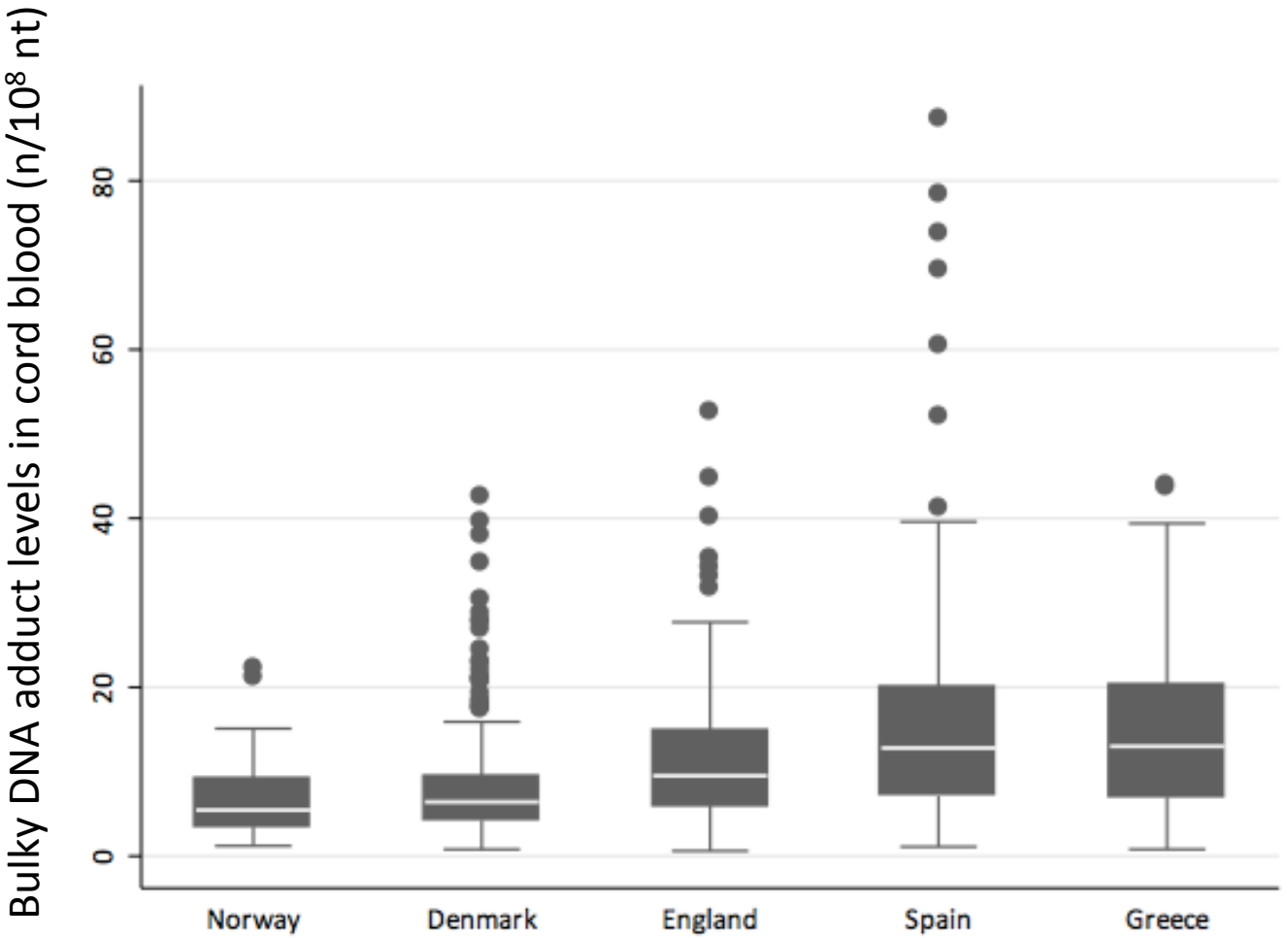
N.a., not available; NO_2, nitrogen dioxide; PM_{10}, particulate matter with an aerodynamic diameter below 10 μm; $PM_{2.5}$, particulate matter with an aerodynamic diameter below 10 μm.

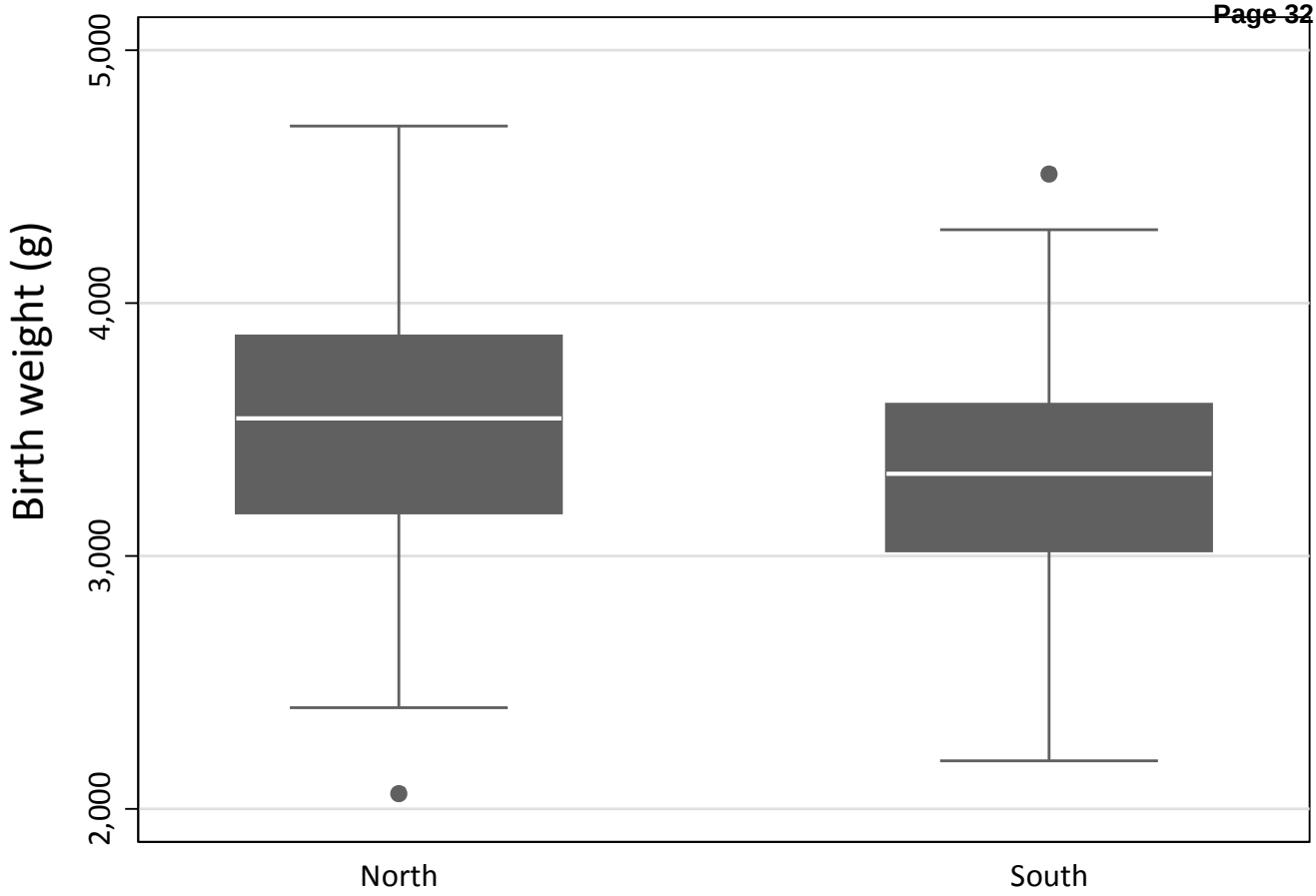
Figure Legends

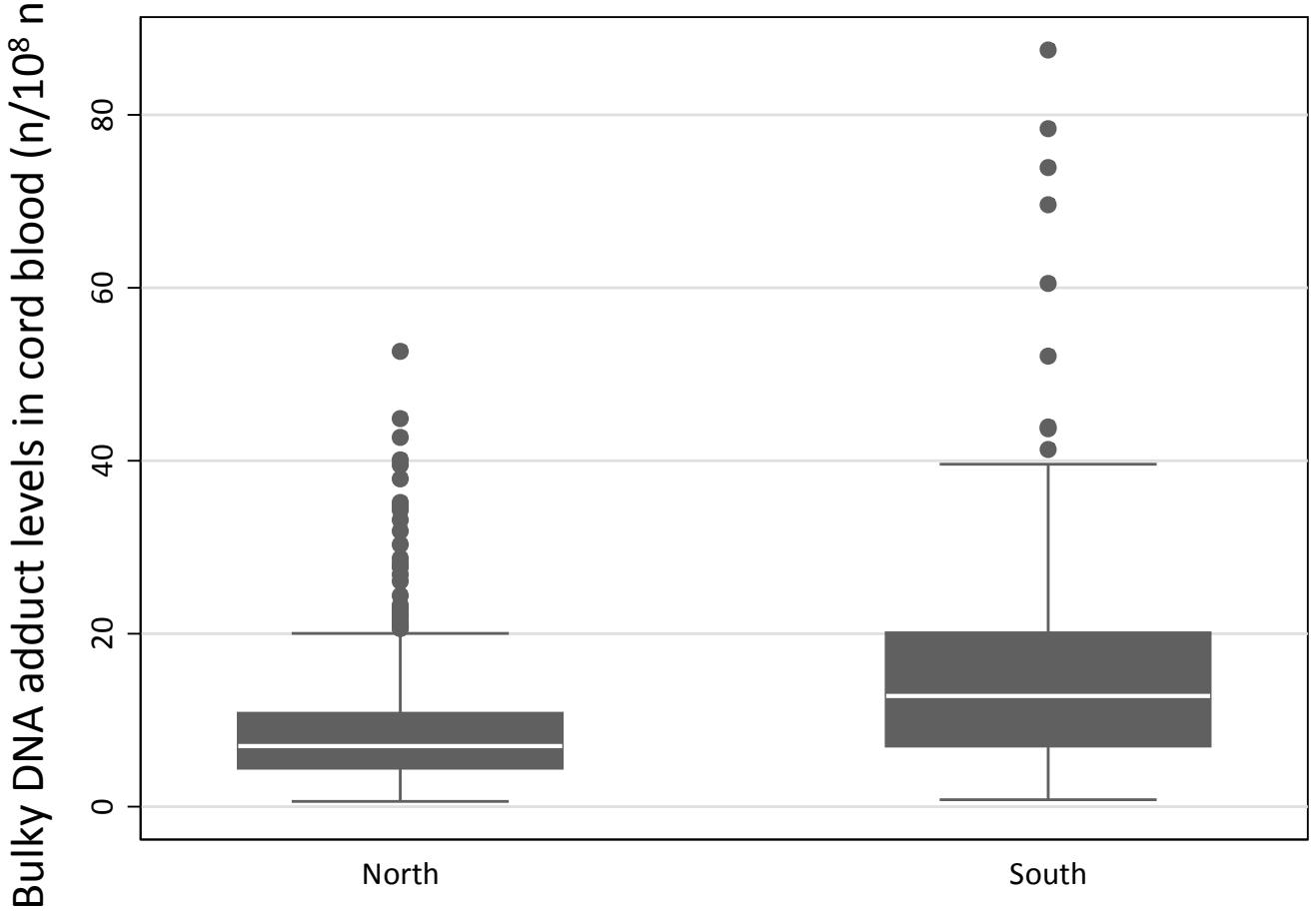
Figure 1. Birth weight (g, top) and bulky DNA adduct levels in cord blood (per 10^8 nucleotides, bottom) - distribution by country.

Figure 2. Change in birth weight (g) associated with the bulky DNA adduct levels in cord blood (per 10^8 nucleotides) by country. Meta-analyses combined effect estimates (random effect of country) of the highest relative to the lowest tertile of cord blood bulky DNA adduct levels further adjusted as described in Table 2 (N=409).


Birth weight (g)
5,000
4,000
3,000
2,000
Norway
Denmark
England
Spain
Greece


Bulky DNA adduct levels in cord blood (n/10^8 nt)
80
60
40
20
0
Norway
Denmark
England
Spain
Greece


Birth weight (g)
5,000
4,000
3,000
2,000
North
South


Bulky DNA adduct levels in cord blood (n/10^8 nt)
80
60
40
20
0
North
South

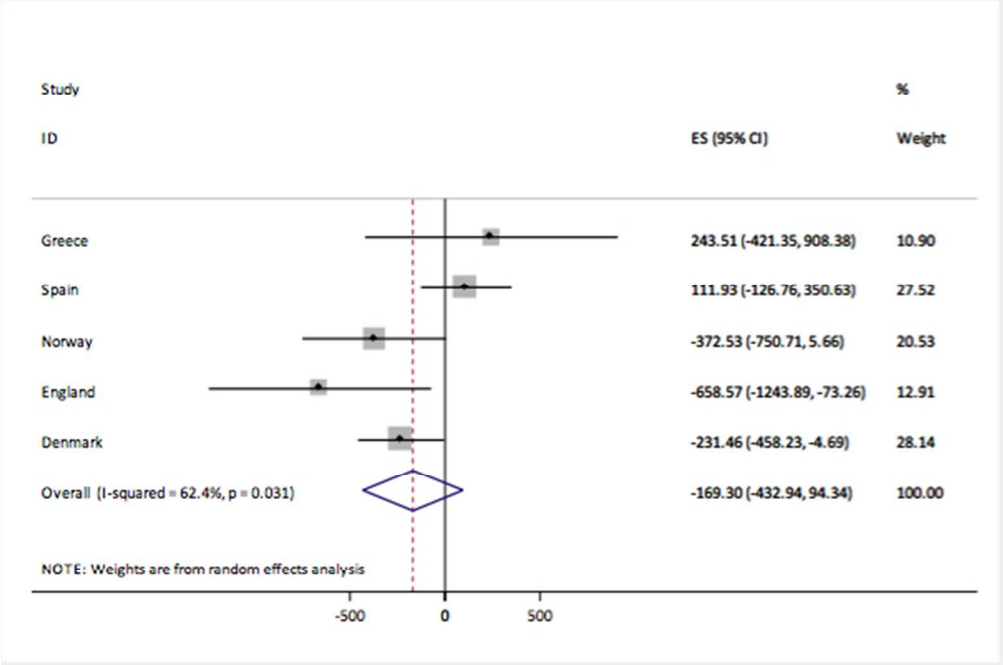


Figure 2

213x141mm (72 x 72 DPI)